Petroleum HPV

201-14901

December 15, 2003

The Honorable Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know
HPV CONSORTIUM
Asphalt Test Plan and Robust Summary

Dear Administrator Leavitt:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Asphalt Test Plan and Robust Summary. Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substances categories and the associated test plans. We are therefore submitting this test plan, as well as the robust summary, directly to EPA to make available for public comment.

Electronic copies of the test plan (in .pdf format) and robust summary (in .pdf format and as an IUCLID export file together with its pdf attachment AD4884.doc) are accompanying this letter via email to the EPA HPV robust summary email address (http://www.epa.gov/chemrtk/srbstsum.htm). This submission is also being sent, via email, to the individuals listed below, including Mr. Charles Auer.

Please feel free to contact me (202-682-8344; twerdokl@api.org) or Tom Gray (202-682-8480; grayt@api.org) with any comments or questions you may have regarding this submission.

Sincerely,

Lorraine Twerdok, Ph.D., DABT
Administrator, Petroleum HPV Testing Program

Cc: C. Auer, USEPA
R. Hefter, USEPA
O. Hernandez, USEPA
Petroleum HPV Testing Group Oversight Committee and Technical Workgroup

201-14901B

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: **ASPHALT**

Summary prepared by: American Petroleum Institute

Creation date: **MAY, 23, 2003**

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product

Remark : Asphalt (Bitumen in Europe) is the residuum produced from the non-destructive distillation of crude petroleum at either atmospheric pressure or under reduced pressure in the presence or absence of steam. Asphalt may also occur as a natural deposit

Asphalts are complex mixtures of hydrocarbons with molecular weights ranging from 500 to 2000. They have high boiling ranges (400-500°C; 752-932°F) and carbon numbers predominantly higher than C25.

Two samples of asphalt that have been used in some of the mammalian toxicity studies were characterized as follows:

Test	API Sample 81-13	81-14
Gravity (°API)	6.6	11.8
Sulfur (wt%)	4.46	0.72
Nitrogen (wt%)	0.51	0.43
Carbon (wt%)	90+	90+
Nickel (ppm)	18	16
Copper (ppm)	<1	<1
Iron (ppm)	33	15
Vanadium (ppm)	39	5
Initial boiling point (°F)	650	662
Aromatic (%)	-	-
Asphaltenes (%)	6.5	1.2

This robust summary does not include any information on studies in man since most of them have been studies designed to assess exposure by biomonitoring methods to bitumen and its fumes during use.

1.13 REVIEWS

Memo : IARC

Remark : IARC reviewed the evidence for the carcinogenicity of bitumen to animals and man and published their evaluation in 1985.

IARC concluded that

> There is sufficient evidence for the carcinogenicity of extracts of steam-refined bitumens, air-refined bitumens and pooled mixtures of steam- and air-refined bitumens in experimental animals.
>
> There is limited evidence for the carcinogenicity of undiluted steam-refined bitumens and for cracking-residue bitumens in experimental animals.

There is inadequate evidence for the carcinogenicity of undiluted air-refined bitumens in experimental animals.

There is inadequate evidence that bitumens alone are carcinogenic to humans.

Subsequently, IARC carried out a further review of newer studies and published their new evaluation in 1987.
In this new review IARC concluded:

Bitumens are not classifiable as to their carcinogenicity to humans (Group 3).

Extracts of steam-refined and air-refined bitumens are possibly carcinogenic to humans (Group 2B).

(34) (35)

Memo : CONCAWE

Remark : CONCAWE reviewed the available information on the health and environmental effects of bitumen and bitumen derivatives.

(27)

2.1 MELTING POINT

Method : Softening Point of Bitumen; ASTM D36

Remark : Asphalts are viscous semi-solid to solid materials at ambient temperatures and do not have sharply defined melting points. They gradually become softer and less viscous as the temperature rises. For this reason, softening points are determined as a means of measuring the flow characteristics under closely defined test conditions. ASTM Standard Method D36 (ASTM 2000) is customarily used to determine the softening points of asphaltic materials. In this method, two horizontal discs of asphalt, each supporting a steel ball are heated under controlled conditions. The softening point is reported as the mean of the temperatures at which the two disks soften enough to allow each steel ball, enveloped in asphalt, to fall a distance of 25 mm (1.0 in.).

Result : Value:

30 - 60 °C	Penetration Grade (CAS No. 8052-42-4)
60 - 75 °C	Hard Grade (CAS No. 8052-42-4)
60 - 130 °C	Oxidized Grade (CAS No. 64742-93-4)

(13) (27)

2.2 BOILING POINT

Value : > 450 °C

Remark : Asphalt and vacuum residue are obtained as the residues from the vacuum distillation of crude oil.

(26)

2.4 VAPOUR PRESSURE

Remark : Asphalt and vacuum residue are obtained as the residues from the vacuum distillation of crude oil. They consist of high molecular weight hydrocarbon molecules having 25 or more carbon atoms. As such they have negligible vapor pressure.

Conclusion Negligible

(26)

2.5 PARTITION COEFFICIENT

Log pow : ≥ 10

Remark : Partition coefficients of various hydrocarbon isomers having 25 carbon atoms were estimated using the computer program EPIWIN (EPA 2000). This range of estimated Log Kow values indicates they are too high to be empirically determined using standard testing methodologies (OECD 1993).

(30) (40)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Method : Dutch Normalisation Institute NEN 7345
Year : 1995
GLP : No data
Test substance : Bitumen/asphalt

Remark : A standardized test was conducted to determine the leaching of polyaromatic hydrocarbon (PAH) compounds from bitumen and asphalt (asphalt defined here as bitumen plus aggregate) materials. Nine different bitumens and an asphalt were tested, covering a representative range of commercially available products. The leaching test consisted of a 140 g layer of bitumen contained in a covered glass dish with purified water having a pH of 4. The liquid/water ratio was fixed at 4.5:1. The asphalt sample was tested as a cylindrical block placed on glass rods in a covered glass dish. The amount of water was chosen to keep the amount of water comparable to that of the tests with the bitumens (4 ml/cm^2). Leachate water was removed for analysis and replace with fresh water after 0.25, 1, 2.25, 4, 9, 16, and 36 days.
In a study similar to Brandt and De Groot (2001), the Asphalt Institute (2003) analyzed 17 polyaromatic compounds in aqueous leachate from fresh hot mix asphalt. Naphthalene was measured at 0.25 µg/l, while all other PAH compounds were below the detection limit (detection limits ranged from 0.015 to 0.194 µg/l). Benzene also was below the detection limit concentration in the leachate (Asphalt Institute 2003).
CONCAWE (2001) states that asphalt and vacuum residue consist of high molecular weight hydrocarbon molecules (m.w. 500 to 15,000) having 25 or more carbon atoms. As such they have extremely low water solubilities. Products are widely used in waterproofing applications.

Result : **Steady state concentrations in leachate water (ng/l)**

Bitumen code/ PAH analysis	**Naphthalene**	**Sum of 2+ rings**
A	35	8.8
B	371	263
C	51	68
D	175	10
E	30	5.9
F	n.v.	51
G	120	17
H	0.9	5.4
I	168	172
Asphalt	33	2.4

n.v. = not valid
The range of bitumens tested showed the same leaching behavior against time. In the first days the concentrations increase and reach steady state between day 3 and day 6.
Generally, only the polyaromatic hydrocarbon (PAH) compounds with 4 rings or less were found in concentrations above 0.1 ng/l. As shown in the above table, naphthalene dominated the concentrations when compared to the PAHs having 3 or more rings.

Reliability : (2) valid with restrictions
A well documented publication which meets basic scientific principles
(10) (23) (26)

3.1.1 PHOTODEGRADATION

Remark : Under ambient conditions, substances in the asphalt and vacuum residue are semi-solid to solid materials having negligible vapor pressure and water solubility. Hence, they do not disperse when released in the environment. However, when used in road-building and roofing applications, these substances may be heated, creating fumes and vapors that could potentially disperse in the atmosphere. Individual constituents in these substances have the capacity to undergo various direct or indirect photodegradation pathways, although the extent to which these substances engage in such reactions depends upon their dispersal and transport where these reactions may take place. For example, polyaromatic compounds can absorb light in the 290 to 800 nm range where direct photolytic reactions can occur, although absorption is not always sufficient to effect a chemical change. Other saturated and mono and diaromatic hydrocarbons have the ability to indirectly photodegrade through interaction with OH or NO3 radicals in the troposphere (Atkinson 1990). Although component hydrocarbons may undergo photodegradation, the physicochemical characteristics of asphalt and vacuum residue under ambient conditions will not facilitate these reactions.

(14)

3.1.2 STABILITY IN WATER

Remark : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. Materials in the asphalt category are not subject to hydrolysis, as they lack these reactive groups.

Reliability : (1) valid without restriction

(33)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Remark : See Section 3.8.

3.5 BIODEGRADATION

Type : Aerobic

Remark : There are no known studies of the biodegradation of bitumen/asphalt using standard guideline methodologies. However, from many years of experience in their use in roadway and roofing applications, they are clearly persistent materials, the absence of biodegradation being a key property (CONCAWE 2001). However, substances in this category are completely

exempt from mechanisms of biodegradation. Various microorganisms have been isolated that are able to utilize asphalt as a source of carbon for growth. For example, Phillips and Traxler (1963) demonstrated that species of Pseudomonas, Chromobacterium, and Bacillus were capable of degrading thin films of asphalt on painted on culture flasks. Degradation between 3 and 25% were measured after one week of incubation, and in one experiment measured 90% after one month. Fluctuations in temperature, pH, and oxygen tension affected to a greater or lesser degree the ability of these microorganisms to biodegrade asphalt (Phillips and Traxler 1963; Cundell and Traxler 1973). Although hydrocarbon components in asphalt appear capable of being biodegraded by specific bacteria, the rate is exceedingly slow and may take decades to effect changes in such materials in commercial use (ZoBell and Molecke 1978). Under realistic exposure conditions where the bulk properties of asphalt limits dispersion and the available surface area for microbial exposure, biodegradation is expected to be minimal.

(26) (28) (42) (48)

3.8 ADDITIONAL REMARKS

Remark : Due to their high molecular weights (C25 and higher) and physicochemical properties, asphalt and vacuum residue will tend to remain intact and within the medium in which they were released (CONCAWE 1992; US EPA 1985). Although substances in this category would not be expected to disperse in the environment, their use in road surfacing and roofing products are widespread. This has generated an interest and concern for the fate and effects of hydrocarbons in fugitive emissions and runoff/leachate during their manufacture and use (NIOSH 2000; NIOSH 2001; Buckler and Granato 1999). Almost exclusively, the interest and concern has been in the content of polyaromatic hydrocarbons generated under these conditions.

Although the vast majority of hydrocarbon molecules are C25 and higher, small amounts of low molecular weight polyaromatic hydrocarbons (PAHs) have been measured in solid matrix materials (API 1987; CONCAWE 1992). While the concentrations of these low molecular weight substances in asphalt and vacuum residue are slight (typically <0.001%) and under normal ambient conditions trapped in the solid matrix, when heated as occurs in road building and roofing applications, asphalt products emit fumes and vapors that contain mixtures of aliphatic and aromatic groups (NIOSH 2000). As fumes and vapors cool, they condense onto local surfaces or collide and stick together with further precipitation from the air (NIOSH 2000), which limits the transport from the site of origin. Vapors of aliphatic and aromatic hydrocarbons which remain suspended have the potential to undergo direct and/or indirect photodegradation in accordance with the molecule's capacity and the conditions that permit those reactions to occur.

Chemical analysis of runoff from "in place" asphaltic materials have found a wide variety of inorganic and organic compounds. However, these substances are attributed to vehicle emissions, spills/droppings of crankcase oil, deicers, nutrients, pesticides/herbicides, fuel additives, maintenance materials and catalytic converter emissions (Buckler and Granato 1999). Bench-scale laboratory leaching studies of fresh bituminous materials have found few measurable quantities of PAHs. In

one such study only trace amounts of naphthalene were found in leachate from fresh asphalt (Asphalt Institute 2003) Brandt and De Groot (2001) also determined that naphthalene dominated the PAHs leached from nine different bitumens, with substantially lesser amounts of 3 and 4 ring PAHs occurring. However, even maximum concentrations did not exceed ng/l levels in the leachate water. In a study of in-place asphalt pavement, samples of weather pavement were brought into the laboratory, crushed, and subjected to leachability trials. That study, of the various PAHs measured, only naphthalene was detected slightly above the detection limit (Asphalt Institute, 2003)

(1) (10) (12) (24) (27) (38) (39) (47)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Remark : See Section 4.9.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Remark : See Section 4.9.

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Remark : See Section 4.9.

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.9 ADDITIONAL REMARKS

Remark : Asphalt and vacuum residue are not expected to cause acute or chronic toxicity to aquatic organisms due to the extremely low water solubility of these materials. This is supported by aquatic toxicity data from other petroleum products having similar types of hydrocarbon constituents (i.e., saturate and aromatic fractions). For example Aromatic Extracts, which contain highly aromatic hydrocarbons of C15 and higher, showed no acute or chronic toxicity in aquatic organisms. Those data were referenced in CONCAWE (2001) and are illustrated in the following table.

Test Species	Value Endpoint	mg/l	Source
Oncorhynchus mykiss	96-H LL_{50}	>1000	BP 1994
Daphnia magna	48-H EL_{50}	>1000	BP 1994
Selenastrum capricornutum	96-H $LL_{50}r$	>1000	BP 1994
	96-H $LL_{50}b$	>1000	BP 1994
Daphnia magna	21-D $EL_{50}S$	>1000	BP 1995
	21-D $EL_{50}R$	>1000	BP 1995

Similarly, lubricating oil basestocks, which contain saturate as well as aromatic hydrocarbons of C15 and higher, showed no acute or chronic toxicity in aquatic organisms. Those data were submitted to the U. S. EPA in support of the Lubricating Oil Basestocks HPV Category (API 2003) as well as referenced in CONCAWE (1997) and are summarized in the

following table.

Test Species	Endpoint	Value, mg/l	Source
Oncorhynchus mykiss	96-H LL_{50}	>1000	BP 1990
Daphnia magna	48-H EL_{50}	>10000	Shell 1988
Selenastrum capricornutum	96-H LL_{50}r	>1000	BP1990
	96-H LL_{50}b	>1000	
Daphnia magna	21-D EL_{50}S	>1000	BP 1995
	21-D EL_{50}R	>1000	

Asphalt and vacuum residue, which contain saturate and aromatic hydrocarbon molecules of C25 and higher, also would not be considered sufficiently water soluble to elicit acute or chronic toxicity in aquatic animals and plants.

Fish hatchery ponds lined with hot-mix asphalt are operated by the Oregon Department of Fish and Wildlife and the Washington State Department of Fisheries who have said to produce millions of high quality fish each year (Asphalt Institute 2003).

(9) (11) (16) (17) (18) (19) (20) (21) (22) (25) (26) (46)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Corn oil
Doses	:	5 g/kg
Year	:	1982
GLP	:	Yes
Test substance	:	Vacuum residue API sample 81-13 (See section 1.1.1.)
Method	:	Test material was administered as a suspension in corn oil to five male and five female Sprague-Dawley rats. Each animal was given a single oral dose of 5 g test material /kg (at a dose volume of 20 ml/kg). The animals were observed for clinical signs at hourly intervals for the first six hours after test material administration and twice daily thereafter. Body weights were recorded before test material administration and again 7 and 14 days after administration. At study termination (day 14) all animals were killed and were subjected to a gross necropsy when any abnormalities observed were recorded.
Result	:	There were no mortalities in the study. Clinical signs included hypoactivity, diarrhea, dark brown and black-stained anal region. Growth was normal during the 14 day observation period. There were no significant treatment-related abnormalities observed at necropsy.
Reliability	:	(1) valid without restriction

(2)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC_{50}
Value	:	> 94.4 mg/m³
Species	:	Rat
Strain	:	Wistar
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Air
Exposure time	:	4.5 hour(s)
Year	:	2000
GLP	:	Yes
Test substance	:	Fume generated from a sample of bitumen condensate
Method	:	Five male and five female Wistar rats (aged approximately 7 wks) were exposed to either clean air (control) or bitumen fume (100mg/m³ as Total hydrocarbon concentration) for 4.5 hours. The extra 30 minutes was necessary in order to achieve the correct exposure concentration for 4 hours. Exposure was by means of a nose-only inhalation system and the animals were individually housed during the remainder of the study. Apart from the exposure period, food and water were available ad libitum. All animals were observed for clinical signs during the exposure period,

several times after the exposure on the same day and daily thereafter.
Records were maintained of the following:

General condition, fur, grooming activity
Visible mucous membranes
Behavior and locomotor activity (lethargy, coma, convulsions, diarrhea and salivation)
Central nervous system symptoms
Breathing pattern
Reflexes (at least 1, 24, 48 hr after cessation of exposure, the following reflexes were assessed - visual placing, climbing reflex, pinna reflex, vibrissae reflex, auditory startle response, pain sensitivity and seizures)
Rectal temperature, once after cessation of exposure.

Body weights were recorded before exposure and again on days 3, 7 and 14. At the end of the study, each animal was subjected to a necropsy.
A t-test was used to determine the statistical significance of differences between treated and control animals for: rectal temperature, body weight and body weight gain.

Result : The exposure conditions are summarized in the following table.

	Clean air control	**Exposure group**
Exposure time	4.5 hrs	4.5 hrs
Temperature	22.7 ± 0.7 °C	23.8 ± 0.5 °C
Humidity	53.3 ± 3.2 %	48.2 ± 2.1 %
Air inflow	20.4 l/min	20.3 l/min
Air outflow	11.8 l/min	8.6 l/min
Conc. THC*	-	65 mg/m³
Conc THC **	-	94.4 ± 7.7 mg/m³
NMAD***	-	85/1.7 nm

* Measured during the 30 minute pre-exposure period
** Measured during the 4 hr exposure period
*** Number median aerodynamic diameter.

No clinical signs of intoxication were observed during or after the exposure period.
No body weight differences were observed.
Body temperature was significantly lower for both males and females at the end of the exposure period.

	Body temperature (°C)	
	Males	**Females**
Control	37.3	37.7
Exposed animals	35.6	36.6

There were no effects on any of the reflexes examined.
There were no gross abnormalities in either the control or treated groups at necropsy.

Test condition : The fume was generated using an evaporation condensation generator.

The bitumen fume condensate was fed via a peristaltic pump to a nitrogen operated dispersion nozzle. A droplet spray was generated and the droplets were evaporated in a heating tube. The hot vapor issued through a nozzle into a slowly flowing cool air stream surrounding the jet. The fume was subsequently diluted with clean air to achieve the intended concentration and the diluted fume was delivered to the nose-only system at a flow rate of about 20 l/min.

Fume concentration was determined by sampling the nose-only unit using

a combination of a glass filter and an XAD absorption tube. The material collected on the filter and the XAD tube was extracted and analyzed separately by IR spectroscopy.
In addition the fume was analyzed once for PAHs.
For continuous monitoring of the total hydrocarbon exposure concentration a flame ionization detector with heated sampling line was used.
Particle size distribution was determined using a scanning mobility particle sizer.

Test substance : The PAH content of the exposure atmosphere was as follows:

PAH	ng/absolute	ng/m³
Naphthalene	6497.56	4709.40
Acenaphthylene	*	*
Acenaphthene	132.41	95.97
Fluorene	58.48	42.39
Phenanthrene	153.59	111.32
Anthracene	58.48	42.39
Fluoanthene	54.25	39.32
Pyrene	131.75	95.49
Benz(a)anthracene	41.36	29.98
Chrysene	42.75	30.99
Benzo(b)fluoranthene	15.27	11.07
Benzo(k)fluoranthene	*	*
Benzo(e)pyrene	31.12	22.56
Benzo(a)pyrene	6.11	4.43
Indeno(1,2,3-cd)pyrene	*	*
Dibenz(ah)anthracene	*	*
Benzo(ghi)perylene	5.84	3.23

Reliability : (1) valid without restriction

(31)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Value : > 2000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 2
Vehicle : None
Doses : 2 g/kg
Year : 1982
GLP : Yes
Test substance : Vacuum residue API sample 81-13 (See section 1.1.1.)

Method : Four male and four female New Zealand White rabbits were used for each dosage level. The skin area designated for treatment was abraded in two males and two females whilst the skin of the other animals remained intact. Undiluted test material was applied to the skin of each rabbit at a dose level of 2000 mg/kg. [The test material was warmed overnight in a water bath to reduce its viscosity].
The treated skin was covered with gauze and an occlusive dressing. The dressings were removed after 24 hours and the treated skin site wiped to remove residual test material.
Collars were fitted to the rabbits throughout the study to prevent ingestion of test material.

Rabbits were observed for clinical signs, hourly for the first six hours after dosing and twice daily thereafter for 14 days.
Body weights were recorded just prior to dosing and again at 7 and 14 days after dosing.
At study termination all animals were killed and subjected to a gross necropsy. Any observed abnormalities were recorded.

Result : After the 24 hour exposure period, it was not possible to remove all of the applied test material due to its tar-like nature.
Mucoid diarrhea was exhibited by one female on day 1 of the study and diarrhea was exhibited by one female on days 6 and 7. No other clinical signs were observed and the growth of the rabbits was normal following dosing.
There were no mortalities and no visible lesions at necropsy.

Reliability : (1) valid without restriction

(2)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : Undiluted
Year : 1982
GLP : Yes
Test substance : Vacuum residue API sample 81-13 (See section 1.1.1.)

Method : Undiluted test material (0.5 ml) was applied to two areas of the skin of six young male New Zealand White rabbits. One area of skin on each rabbit had been abraded whilst the other was intact. The treated skin sites were covered with an occlusive dressing which remained in place for 24 hours.
Body weights were recorded prior to material application and at weekly intervals throughout the study.
After the 24 hours exposure, the coverings were removed and the skin was wiped from the area as thoroughly as possible without irritating the skin. A record was made of the degree of erythema and edema (using the Draize scale) immediately after dressing removal and again at 72 hours, 96 hours, 7 and 14 days.
At study termination all animals were killed and subjected to a gross necropsy. Any observed abnormalities were recorded.

Result : The primary dermal irritation scores* were:

Observation	**Erythema**		**Edema**	
	Intact	**Abraded**	**Intact**	**Abraded**
24 hrs.	0.2	0	0	0
72 hrs.	0	0.2	0.2	0.2
96 hrs.	0	0.02	0.2	0.2
7 days	1	1	0.3	0.2
14 days		0.8	0.8	0 0

Primary dermal irritation index**: 0.2

* Primary dermal irritation score is the sum of the irritation scores for each site divided by the number of animals at each observation period.

** Primary dermal irritation index is the sum of the 24 and 72 hour primary dermal irritation scores for intact and abraded skin (8 values) divided by 4 and rounded to the nearest tenth.

Growth was unaffected by treatment and there were no visible lesions at necropsy.

Reliability : (1) valid without restriction

(2)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Comment : Rinsed after 30 seconds for 3 rabbits. Eyes not rinsed for 6 rabbits
Number of animals : 9
Vehicle : None
Year : 1982
GLP : Yes
Test substance : Vaccum residue API sample 81-13 (See section 1.1.1.)

Method : 0.1 ml of undiluted test material was placed into the conjunctival sac of one eye of each of nine rabbits. The eyelids were held together for one second to prevent loss of test material. 30 seconds after instillation of the test material the eyes of three rabbits were flushed with lukewarm water for one minute. Body weights were recorded just prior to test material instillation and weekly thereafter throughout the study.
Eyes were examined for ocular lesion 1, 24, 48, and 72 hours and 7 days after treatment. Scoring of lesions was according to the Draize scale and was recorded for each observation time. Sodium fluorescein and an ultraviolet light was used to assist in the examination of the cornea for possible damage at the 72 hour and 7 days observation times. At study termination all animals were killed and were subjected to a necropsy. Any abnormalities were recorded.

Result : The primary eye irritation scores* were:

Score	Unwashed eyes (mean of 6 rabbits)	Washed eyes (mean of 3 rabbits)
1 hour	2.0	1.3
24 hours	4.0	5.3
48 hours	4.2	2.0
72 hours	1.8	0.7
7 days	0	0

* Primary eye irritation score is the total eye irritation score for all animals, divided by the number of animals in eachgroup at each observation period (ie average irritation score).

One rabbit exhibited hypoactivity, and was possibly anorexic. It had a bloated appearance and diarrhea at the 7 day observation time. These clinical signs were not considered to be treatment-related. With the exception of the animal referred to above, body weights were normal throughout the study and there were no abnormalities observed at necropsy.

Reliability : (1) valid without restriction

(2)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	1st: Induction undiluted occlusive epicutaneous 2nd: Challenge undiluted occlusive epicutaneous
Number of animals	:	10
Vehicle	:	None
Result	:	Not sensitizing
Year	:	1984
GLP	:	Yes
Test substance	:	Vacuum residue API sample 81-13 (See section 1.1.1.)

Method :

A group of ten young adult male guinea pigs were used for this study.
0.4 ml of test material was applied to the shorn dorsal skin of the guinea pigs using Hilltop chambers. The applied material was covered with an occlusive dressing. After six hours the patch was removed and any residual test material was removed from the skin using liquid paraffin as a solvent.
The animals received one treatment each week for three weeks.
Two weeks following administration of the third dose, a challenge dose of test material was applied to a virgin skin site on the opposite flank of the animal. This test site was occluded as before.

24 and 48 hours after each skin application an assessment of reaction to the dose was made and recorded.

The positive control group (20 animals) were treated in a similar manner to the animals in the test group except that 2,4-dinitrochlorobenzene was used at a concentration of 0.3% in 80% ethanol for the sensitizing doses. The challenge dose of positive control was 0.1% in acetone.

A group of 10 animals was used as naive controls. This group of animals received challenge dose only.

In a previously conducted range finding study, it was established that the test material should be administered undiluted for both sensitizing and challenge doses.

The criteria for evaluating the response:
Determination of sensitization was based on reactions to the challenge dose.
Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1 are seen in the naive control animals.
If grades of 1 or greater are noted in the naive control animals, then the reactions of the test animals that exceeded the most severe naive control reactions are considered sensitizing reactions.

Result : No skin reactions were observed in any of the naive control animals or in the animals in the test group.
In contrast, skin reactions 1 or greater for erythema were observed in 17/20 animals and for edema in 8/20 animals.
These data demonstrate that the test material was not sensitizing.

Reliability : (1) valid without restriction

(5)

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	1st: Induction undiluted occlusive epicutaneous 2nd: Challenge undiluted occlusive epicutaneous
Number of animals	:	10
Vehicle	:	None
Result	:	Not sensitizing
Year	:	1984
GLP	:	Yes
Test substance	:	Vacuum residue API sample 81-14(See section 1.1.1.)
Reliability	:	(1) valid without restriction

(6)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	Rabbit
Sex	:	Male/female
Strain	:	New Zealand white
Route of admin.	:	Dermal
Exposure period	:	6 hours
Frequency of treatm.	:	Once per day, three times each week for four weeks
Doses	:	200, 1000 & 2000 mg/kg/day
Control group	:	Yes
Year	:	1983
GLP	:	Yes
Test substance	:	Vacuum residue API sample 81-13 (See section 1.1.1.)

Method : Groups of five male and five female young adult New Zealand White rabbits were used for this study. The dose groups employed were: control, 200, 1000 and 2000 mg/kg/day

Application was by weighing the appropriate quantity of undiluted test material onto a 4x4 inch patch which was applied to the shorn dorsal skin of each rabbit. The patch was covered by an occlusive dressing. Six hours after administration of the test material, the patches were removed and any residual test material was removed from the skin by gentle wiping with a dry gauze.
This procedure was repeated once daily, three times weekly until a total of 12 applications of test material had been made. Sham-treated controls underwent the same procedure except that no test material was applied. Clinical observations were made twice daily. Body weights were recorded just before the first application of test material and once weekly throughout the study thereafter. The skin exposure site was examined and reactions recorded daily during the test period. Degree of erythema and edema were assessed using the standard Draize method.

At study termination, blood samples were taken from the animals for the following hematological and clinical chemical determinations.

Hematology	**Clinical chemistry**
Erythrocyte count	Glucose
Total leukocyte count	Blood urea nitrogen
Differential leukocyte count	Alkaline phosphatase
Hemoglobin	SGOT
Hematocrit	SGPT

RBC morphology Total protein

All animals were then sacrificed and underwent a gross necropsy.
The following organs were weighed
Heart, Liver, spleen, kidneys, thyroid, pituitary, testes, ovaries and brain.

The following tissues were removed, preserved and prepared for histological examination.

Heart, lungs, bronchi, trachea, thyroid, parathyroids, cervical lymph nodes, salivary gland, tongue, esophagus, stomach, duodenum, jejunum, ileum, sacculus rotundus, colon, thymus, spleen, liver, pancreas, kidneys, adrenals, vagina, seminal vesicles, testes/ovaries, epididymides, prostate/uterus, mesenteric lymph nodes, urinary bladder, adipose tissue, mammary gland, brain (cerebrum, cerebellum, pons), pituitary, spinal cord (two sections), skeletal muscle, sciatic nerve, skin (treated and untreated), bone, bone marrow, eyes, gross lesions.

Statistical analyses
Body weights, clinical pathology and absolute and relative organ weight data of the control and treated groups were statistically compared using a two-tailed Student's t-test at the 5% probability level.

Result : Two animals died and two were sacrificed moribund during the study but none of these was considered to be compound-related.
Treatment-related clinical signs in animals that survived to day 28 included: thin appearance, decreased food intake, flaking skin and wheezing.

Erythema for animals exposed to test material could not be scored at most daily intervals because the test material could not be removed from the skin, thus obscuring the test site.
Edema was recorded in all groups except controls throughout the study.
The severity ranged from very slight to slight.
The average total edema score for each group was as follows:

	Male	**Female**
Control	0	0
200 mg/kg/day	27	18
1000 mg/kg/day	31	36
2000 mg/kg/day	37	38

A treatment-related suppression in body weight gain was recorded for the high dose male groups.
The total weight gains (kg) over the course of the study are shown in the following table

	Male	**Female**
Control	0.6	0.5
200 mg/kg/day	0.6	0.4
1000 mg/kg/day	0.4	0.5
200 mg/kg/day	0.1*	0.4

* P< 0.05%

There were no treatment-related trends in any of the hematological or clinical chemical parameters that were measured. Statistical analyses revealed differences between controls and the following groups. Although the differences for RBC and glucose were not regarded as treatment-

related, the significance of the changes in alkaline phosphatase was not understood.

Parameter	Dose group	Sex	Difference
RBC	200 mg/kg/day	M	+ 12%
Alk Phos.	2000 mg/kg/day	M	- 50%
Glucose	200 mg/kg/day	F	- 16%

There were significant differences in the following, all of which were considered to be incidental and not treatment-related.

1000 mg/kg/day		
Males	Absolute left kidney weight	- 14%
2000 mg/kg/day		
Males	Absolute left kidney weight	- 16%
Males	Absolute/relative right adrenal weight	+ 86/133%
Females	Absolute pituitary weight	+ 63%
Females	Relative spleen weight	+ 50%

Treatment-related gross necropsy findings were confined to the skin. In these cases the skin was reddened and thickened.
Treatment-related microscopic findings were also confined to the skin. Minimal to moderate subacute acanthotic dermatitis and minimal to moderate hyperkeratosis was observed in the high dose males and females (5/5 males, 3/5 females). Females appeared more severely affected.

Incidental findings were observed and were consistent with Encephalitozoon infection.

Reliability : (1) valid without restriction

(3)

Type : Sub-acute
Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 6 hours
Frequency of treatm. : Once per day, three times each week for four weeks
Doses : 200, 1000 & 2000 mg/kg/day
Control group : Yes
Year : 1983
GLP : Yes
Test substance : Vacuum residue API sample 81-14 (See section 1.1.1.)

Result : The results of this study were similar to those described above with sample 81-13, except that there were no reductions in body weight gain in any of the treated groups.

Reliability : (1) valid without restriction

(4)

Type : Sub-chronic
Species : Rat
Sex : Male/female
Strain : Wistar
Route of admin. : Inhalation
Exposure period : 6 hours per day
Frequency of treatm. : 5 days per week for 14 weeks

Doses	:	4, 20 & 100 mg/m³
Control group	:	Yes
NOAEL	:	20 mg/m³
LOAEL	:	100 mg/m³
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	2001
GLP	:	Yes
Test substance	:	Bitumen fume from bitumen condensate

Method :

Groups of sixteen Wistar rats of each sex (approximately 7 weeks of age) were exposed either to clean air or bitumen fumes at concentrations of 4, 20 or 100 mg/m³. Exposures were by nose only for six hours each day, five days a week for 14 weeks. The animals were individually housed with free access to food and water in between exposure periods.
All animals were observed daily for clinical signs. Additionally all animals were removed from their cages once each week and were examined for abnormalities. Body weights and food intakes were recorded weekly starting before exposure to test material had begun.

Of the 16 animals in each group, 10 were designated for the 90 day study and six for Broncho alveolar lavage (BAL).

At the end of the study, animals were fasted overnight and were then killed and subjected to a detailed post-mortem examination. Blood samples were taken for the following clinical chemical and hematological examinations.

Clinical chemistry	Hematology
Aspartate aminotransferase	Erythrocyte count
Alanine aminotransferase	Hemoglobin
Gamma glutamyl transferase	Mean erythrocyte volume
Alkaline phosphatase	Mean erythrocyte hemoglobin
Total bilirubin	(mass and concentration
Urea	Total leukocyte count
Creatinine	Differential leukocyte count
Total protein	Platelet count
Albumin	Prothrombin time
Cholesterol	
Glucose	
Sodium	
Potassium	
Calcium	
Chloride	
Inorganic phosphate	

Globulin and albumin/globulin ratios were also calculated

Urine was collected prior to sacrifice for the following semi quantitative analyses: leukocytes, pH, protein, glucose, ketones, bilirubin, blood, nitrate and uribilinogen. Osmolality was measured quantitatively.
The following organs were weighed at necropsy: Lung (including 2/3 of trachea, liver, adrenals, kidneys and testes. Relative organ weights were calculated.
The following tissues were collected from each rat and fixed for subsequent histopathology.
Brain, pituitary, tongue, eyes, lacrimal glands, Harderian glands, nasal and pharyngeal cavities, larynx, pharynx, trachea, thyroid, parathyroids, lungs, thymus, heart, aorta, lung associated lymph nodes, salivary glands, mandibular lymph nodes, liver, pancreas, spleen, kidneys, adrenals, esophagus, forestomach, duodenum, jejunum, ileum, cecum, colon,

rectum, mesenterium and lymph nodes, urinary bladder, testes, epididymis, prostate, seminal vesicles, ovaries, uterus, vagina, mammary glands, skeletal muscle, femur with bone marrow and joint, spinal cord, peripheral nerve (N. ischiadicus) and sternum with bone marrow.

A bronchoalveolar lavage (BAL) was performed on six rats from each group and the cell concentration was determined using a counting chamber. Cytoslides were prepared from the lavagate for differential cell count (macrophages, PMNs, lymphocytes).
After centrifugation of the lavage fluid the supernatanat was used for the determination of some relevant biochemical indicators of lung damage.
The following parameters were measured:
cell number, differential cell count, total protein, lactic dehdrogenase, ß-glucuronidase and gamma-Glutamyl-transferase

Formalin-fixed terminal bronchioles and lung parenchymal cells were examined for cell proliferation using the sensitive S-phase response method. Proliferating cells were labeled by 5-bromo-2'-deoxyuridine (BrdU) which was administered to five animals per group by a minipump following 90 days of inhalation. The animals were kept for additional seven days without inhalation of test material until sacrifice.
The rats were anesthetised and on the back of the rats an area of 5 x 10 cm was shaved. The area was disinfected and the skin was cut to allow implantation of the minipump. After implantation, the incision was closed and disinfected again.
The lung slides were prepared and stained immunohistochemically following denaturation of the DNA (antibody technique). The slides were evaluated by analyzing an appropriate number of cells from the proximal regions of the pulmonary parenchyma for each rat. For an appropriate number of airways, the unit length labeling index was estimated.

Statistical evaluation
Statistical tests on the comparison of treatment groups were performed at the level of P = 0.05
Body weight, food and water consumption, hematology and clinical chemical data were analyzed using analysis of variance as a global test.
Pairwise comparisons of the means of the treatment groups with the controls were performed using the Dunnet's modification of the t-test.
For comparisons between two treatment groups, the two sided t-test at a level of P = 0.05.
Evaluation of histological findings: significance of differences of the frequencies were evaluated as pair wise comparison between clean air control and treatment groups using Fisher's exact test. These test were performed at a level of P = 0.05.

Result : The mean fume concentrations (total hydrocarbon content) and proportions of vapor and fume in the exposure chambers were:

Nominal concentration (mg/m³)	**Actual concentration (mg/m³)**	**Particulate/ vapor (%)**	**Particle* size NMAD (nm)**
4	5.53	24.6/75.4	105
20	28.17	42.9/57.1	82
100	149.17	68.1/31.9	86

* NMAD = number median aerodynamic diameter

No clinical signs of intoxication were observed. There was one mortality but this was not treatment-related.

Body weights in the 100 mg/m³ males became apparent after one week of treatment and the difference increased during the study. At the end of the study the males in this group weighed 10% less than the corresponding controls.
Milder effects on body weight were noted in all female groups (-5%) exposed to bitumen fumes. Food consumption was also less in the 100 mg/m³ group males and this correlated with the reduced body weights. Water consumption was unaffected by treatment.

There were no toxicologically relevant findings in the hematological parameters measured.

In the 100 mg/m³ males the following differences were recorded in the clinical chemistry values:

- 20% Increase in mean urea
- 8% Increase in mean potassium
- 3% Decrease in calcium concentration

A 3% decrease in calcium concentration was also recorded for the 20 mg/m³ males
No other treatment-related changes were noted in the clinical chemical evaluations.

There were no differences in the urinalysis data.

There were small changes in the data from the BAL evaluations. These were as follows:

100 mg/m³ males

- 93% increase in lactic dehydrogenase
- 53% increase in gamma glutamyl transferase

100 mg/m³ females

- cell concentration increased by 20%

4 mg/m³ males

- 42% increase in gamma glutamyl transferase

There were no treatment-related findings in organ weights at necropsy. However, there was a 7.6% higher relative kidney weight in the 100 mg/m³ males. This was attributed to the decreased body weights in this group.

Gross Pathology
There were no treatment-related gross abnormalities at necropsy.

Histopathology
The following treatment-related observations were recorded.

Nasal and paranasal cavities
Changes were only observed in the 100 mg/m³ groups.
The changes consisted of

- Very slight to moderate eosinophilic cytoplasmic inclusions (hyalinosis) observed exclusively in epithelial cells of 8/10 males and 10/10 females. This degenerative lesion affected the respiratory epithelium with olfactory involvement occurring primarily near the olfactory/respiratory transition area.

  Occasionally, eosinophilic cytoplasmic inclusions were also seen in

cells of the submucosal nasal glands. 1/10 females had moderate multifocal eosinophilic hyalinization of the submucosalglands.

There was focal/multifocal very slight to moderate mucous cell hyperplasia associated with the hyalinosis. Incidences were 10/10 males, 9/10 females compared to 1/10 males of the control group.

Very slight to slight multifocal mucosal inflammatory cell infiltration was observed in 4/10 males and 3/10 females.

Kidneys

The incidence of multifocal very slight to slight tubular basophilia was markedly increased in 8/10 males compared to controls or other bitumen treated groups (4-5/10 per group). This finding was not statistically significant, but a treatment-related effect cannot be excluded.

Other degenerative changes such as tubular cell degeneration, interstitial mononuclear cell infiltration and interstitial fibrosis occurred at incidences between 1/10 and 3/10 per group, but were also more common in groups exposed to bitumen fumes.

There were no other treatment-related histological changes in any other organ examined.

Results of pulmonary labeling studies with BrdU
There were no statistically significant differences for the parameters measured in the labeling studies.
However, the mean parenchymal labeling indices were slightly elevated in the males of the 20 and 100 mg/m³ groups compared to controls. In the female groups the labeling indices were higher in all the treated groups compared to controls.

Group	Parenchymal labeling index
Control	0.99
4 mg/m³	0.87
20 mg/m³	1.14
100 mg/m³	1.32

Test condition : The fume was generated using an evaporation condensation generator.

The bitumen fume condensate was fed via a peristaltic pump to a nitrogen operated dispersion nozzle. A droplet spray was generated and the droplets were evaporated in a heating tube. The hot vapor issued through a nozzle into a slowly flowing cool air stream surrounding the jet. The fume was subsequently diluted with clean air to achieve the intended concentration and the diluted fume was delivered to the nose-only system at a flow rate of about 35 l/min.

Fume concentration was determined twice per week during the first week and weekly thereafter by sampling the nose-only unit using a combination of a glass filter and an XAD absorption tube. The material collected on the filter and the XAD tube was extracted and analyzed separately by IR spectroscopy.
In addition the fume was analyzed once each week for PAHs.
For continuous monitoring of the total hydrocarbon exposure concentration a flame ionization detector with heated sampling line was used.
Particle size distribution was determined 16-18 times using a scanning

mobility particle sizer.

Test substance : The mean PAH concentrations in the fumes for the various treatment groups were as shown in the following table.

	Mean concentration (ng/m³)			
PAH	**100**	**20**	**4**	**Control**
Naphthalene	8304	1641	409	232
Acenaphylene	nq	nq	nq	7.37
Acenaphthene	4754	1046	222	31.9
Fluorene	11162	2296	505	33.1
Phenanthrene	15743	2450	449	22.4
Anthracene	nq	nq	nq	nd
Fluoranthene	631	150	26.2	1.65
Pyrene	1311	303	57	nd
Benzo(a)anthracene	217	45.8	7.86	nd
Chrysene	377	77.6	13.2	0.90
Benzo(b)fluoranthene	116	23.1	4.73	nd
Benzo(k)fluoranthene	nd	nd	nd	nd
Benzo(e)pyrene	222	45.5	8.8	nd
Benzo(a)pyrene	53.5	10.4	1.98	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd
Dibenzo(a)anthracene	21	2.49	nd	nd
Benzo(ghi)perylene	49.9	9,83	1.82	nd

nd = not determined
nq = not quantified

Reliability : (1) valid without restriction

(32)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : S. typhimurium, strains TA98 & TA100
Metabolic activation : With and without
Year : 1987
GLP : No data
Test substance : Penetration bitumen (3 samples)

Method : DMSO extracts of the bitumen samples were tested at increasing doses by means of the Ames test, using TA98 and TA100 strains, with and without rat-liver enzyme system (±S9 mix).

The bitumen samples were also separated into four fractions by liquid chromatographic separation and these fractions were also tested in the Ames test. However, since it had been reported previously that petroleum distillates may have inhibitory effects on mutagenic activity, the derivatives in this study were tested in the presence of an increased concentration of S9 (50% instead of 10%).

The ether and acetone extract of the fume samples were dissolved in DMSO. These solutions were tested as described above. Blank extracts of unloaded filters were also tested.

[It should be noted that the above summary contains all the information provided in the publication. No other experimental details were provided].

Result : The results of the mutagenicity studies are given in the following table.

Sample	DMSO *	Bitumen ext. **		TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
		Solid bitumen samples					
1		0.1	1.3	23±2	44±5	106±15	143±19
		5.0	65.2	34±5	62±9	165±24	207±31
2		0.1	1.1	22±3	36±4	127±16	138±18
		5.0	56.3	35±4	45±6	10±18	182±15
3		0.1	1.0	18±3	61±11	134±26	206±22
		5.0	43.3	37±4	50±7	145±18	167±19
Negative control (DMSO)				19±4	33±8	120±10	150±20
Positive control***				572±41	261±31	1358±91	896±78
		Bitumen fume samples					
Ethyl ether extracts							
S1		0.1	0.2	23±2	29±4	119±14	148±20
		6.0	12.5	15±3	55±10	120±30	138±17
S2		0.1	0.2	20±4	31±12	112±15	135±7
		6.0	12.3	31±6	36±4	131±12	129±11
Acetone extracts							
S1		0.05	5.0	19±6	25±2	105±19	137±22
		0.2	20.0	15±7	23±7	110±13	122±9
S2		0.05	15.1	17±4	24±3	97±10	11924
		0.2	60.0	16±2	22±6	104±12	140±23
Negative control (DMSO)				16±3	28±4	109±15	138±19
Positive control***				531±82	280±44	1402±127	820±91

* DMSO extract residue (mg/plate)
** Corresponding dose of bitumen or airborne particulate (mg/plate)
*** Positive controls are:

TA98-S9	2-nitrofluorene (1µg)
TA98+S9	benzo(a)pyrene (1µg)
TA100-S9	sodium azide (1µg)
TA100+S9	benzo(a)pyrene (1µg)

The authors concluded that neither the solid bitumen samples nor the bitumen fume samples were mutagenic, with or without S9 activation, in the assays conducted.

Test substance : Three different samples of solid penetration bitumens (80 to 100 penetration grade) were collected from road paving operations.
The bitumen samples were dissolved in benzene. Asphaltenes were separated from the samples by precipitation with n-heptane. The heptane-soluble substances were weighed to constant weight and submitted to extraction with dimethylsulfoxide (DMSO), which concentrates mainly PAH.
The DMSO extracts were divided in two, one half was used for PAH analysis and the other half was used for mutagenicity testing.
The results of an analysis of PAH content of the samples is shown in the following table.

PAH	Concentration (µg/g) Sample 1	2	3
Naphthalene	28.7	-	-
Acenaphthylene	-	-	-
Acenaphthene	2.1	3.4	3.7
Fluorene	-	1.0	1.2
Phenanthrene	5.5	14.3	11.2
Anthracene	3.1	-	7.3
Fluoranthene	24.3	31.0	40.0
Pyrene	-	10.9	8.3
Benzo(a)anthracene	10.1	-	5.0
Chrysene	50.6	35.0	72.0
Benzo(b)fluoranthene	-	29.0	36.3
Benzo(k)fluoranthene	3.4	9.1	8.4
Benzo(a)pyrene	2.1	13.1	7.1
Benzo(ghi)perylene	2.7	4.5	1.9
Dibenzo(a,h)anthracene	3.2	5.4	8.6
Indeno(1,2,3-cd)pyrene	1.4	2.1	7.1
TOTAL PAH	137.2	158.8	218.1

- = Not detected

In addition, two bitumen fume samples were collected by high-volume sampler and glass filters (Gelman).
The first sampling (S1) was performed during loading and pouring operations for two consecutive hours. The second sampling (S2) was performed for two hours but only during the periods of bitumen exposure. The filters were sonicated first with ethyl ether (30 mins.) and then with acetone (30 mins.). The ether extracts were divided into two portions, one for mutagenicity testing, the other for analysis.
The results of the analysis were as follows:

PAH	Concentration (µg/m^3) Sample 1	2
Naphthalene	0.18	0.24
Acenaphthylene	-	-
Acenaphthene	0.16	1.26
Fluorene	0.02	0.08
Phenanthrene	0.06	0.22
Anthracene	0.03	0.13
Fluoranthene	0.39	1.13
Pyrene	0.35	0.54
Benzo(a)anthracene	0.54	3.50
Chrysene	0.16	0.20
Benzo(b)fluoranthene	-	1.03
Benzo(k)fluoranthene	0.09	0.67
Benzo(a)pyrene	0.03	0.61
Benzo(ghi)perylene	0.01	0.19
Dibenzo(a,h)anthracene	0.03	0.98
Indeno(1,2,3-cd)pyrene	0.02	0.05
TOTAL PAH	2.10	9.70

- = Not detected

Reliability : (2) valid with restrictions
Although the description of the assay was not complete, the authors cited Ames as the method used.

(37)

Type : Ames test
System of testing : S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100
Metabolic activation : With and without
Year : 1984
GLP : No
Test substance : Four samples of asphalt in xylene

Method : A standard plate assay was used.
Strains of S. typhimurium were TA 1535, TA1537, TA 1538, TA 98 and TA 100.
Assays were carried out in the presence and absence of a rat liver microsomal activation system. The activation assays included 50 µl of S-9 fraction per plate as well as the required co-factors.
Assays were conducted at six dose levels of the asphalt paint: 0.005, 0.01, 0.1, 1.0, 5.0 and 10.0 µl per plate.
These concentrations were attained by adding dilutions of the paints in DMSO at a constant volume of 50 µl per plate.
Negative (solvent) and positive controls were assayed concurrently with each test sample.
The positive controls in assays without S-9 activation were sodium azide for TA1535 and TA 100, 2-nitrofluorene for TA 1538 and TA 98 and 9-aminoacridine for TA 1537. For the activation assays, 2-aminoanthracene was used for all five strains.

The criteria for assessing the response were the observation of twice the number of histidine-independent revertants per plate and a dose-related increase in the response. Mutagenic activities were quantitated by estimated using the number of revertants per µl of sample from the linear portion (initial slope) of the dose-response curves.

Result : No toxicity was evident in the assays conducted with the asphalt paints. None of the asphalt paint samples were found to be mutagenic either in the absence or presence of S-9 activation.

Test substance : Four asphalt paint samples were used. They were composed of a bitumen cutback to which xylene was added in small quantities - see below.

The asphalt cutbacks were derived from petroleum asphalt cut back to 64% solid with mineral spirits

Sample	Component	% w/w
Asphalt A		
	Asphalt cutback	89
	Xylene	1
	Mineral spirit	10
Asphalt B		
	Asphalt cutback	98
	Xylene	2
Asphalt C		
	Asphalt cutback	97
	Xylene	3
Asphalt D		
	Asphalt cutback	97
	Xylene	3

The PAH contemt of samples A and D were:

PAH	Concentration of PAH (mg/g) Sample A	Sample D
Naphthalene	0.2	0.3
Biphenyl	<0.01	>0.01
Acenaphthalene	<0.01	>0.01
Acenaphthene	ND	ND
Fluorene	<0.01	<0.01
9-H-Fluorene	<0.01	ND
Phenanthrene	<0.01	<0.01
Anthracene	<0.01	ND
Acridine	<0.01	<0.01
2-Methylphenanthrene	<0.01	<0.01
2-Methylanthracene	ND	
Fluoranthene	ND	ND
Pyrene	ND	ND
1-Methylpyrene	ND	ND
Chrysene+ benzo(a)anthracene	ND	ND
Benzo(a)pyrene + benzo(e)pyrene	ND	ND

Reliability : (2) valid with restrictions
It is doubtful that the study was conducted according to GLP. Nevertheless the study was reported fully, thus allowing a critical appraisal.

(45)

Type : Ames test
System of testing : S. typhimurium TA98
Metabolic activation : With
Year : 1993
Test substance : Fume condensates of coal tar pitches, roofing asphalts and paving asphalts

Method :

<u>Fume generation</u>
Fumes of the test material were generated in the laboratory.
Fumes of the roofing asphalts and coal tar pitches were generated by heating 10 kg samples to 232 or 316 °C for 6 hours. The samples were stirred at 200 rpm and air was passed over the materials at a rate of 10 liters per minute. Fumes and vapors were condensed in a series of traps. After each run, the condensates from all traps were combined and weighed. The material obtained consisted of oil and aqueous phases and the oil phase was separated and used in this study as fume condensate.

Paving asphalt fumes were generated in a similar manner except that air was not passed over the material and the material was only heated to 163 °C (one sample was heated to 221 °C).

<u>Preparation of DMSO extracts of condensates</u>
DMSO extracts were prepared by heating a 200 mg/ml mixture of the condensate and DMSO at 60 °C for 1 hour with agitation at 150 rpm. After incubation, the samples were centrifuged at approximately 1000 rpm for 5 minutes at 22 °C. The DMSO layer was removed and used for testing.

<u>Ames test</u>
This was performed using Salmonella typhimurium TA98 using the

Blackburn modification (Blackburn et al, 1984, 1986) of the Ames test (Ames et al, 1975).
The modified test system was used because asphalt fume condensates are very similar to water-insoluble petroleum distillates which exhibit low mutagenic activity in the standard Ames test.
The modified system used DMSO extracts of the test material. All media and solutions were prepared according to the methods described originally by Ames et al. Metabolic activation was provided by Aroclor-induced hamster liver enzymes (S9). The final concentration in all assays was 400 µl/plate in order to optimize metabolic activation of PAHs in the samples. All concentrations were plated in triplicate. Testing was conducted using a pre-incubation assay in which the bacteria, test material and S9 were pre-incubated at 37 °C with shaking for 20 minutes before being plated. The plates were incubated for 48 hours at 37 °C and mutant colonies were counted.

The positive control was a commercial No 6 residual fuel oil containing vanadium and nickel. In each assay the positive control was tested at a concentration of 50 µl/plate.

If a dose-related doubling of the mean mutant count (relative to the mean solvent control) was reached, the material was considered to be mutagenic.
Non-linear regression was used to determine the slope of the initial linear portion of the dose-response curve. This value was used as an index of mutagenicity, or mutagenicity index (MI). When more than one experiment was conducted the MIs were pooled and an MI for the pooled data was calculated.

PAH analysis
Quantitative determination of the concentrations of 16-18 individual PAH was performed using EPA method 8310.

Result **:** The results are shown in the following table.
Values shown are slope of dose response curve (± asymptotic standard error).
All positive control responses were stated to be within the expected range.

Sample No. (description & generation temperature)	**PAH* content ppm**	**Mutagenicity index Individual experiments**			**Pooled data**
Coal tar pitch					
1-a (232°C)	4529	725(35)			
1-b (316°C)	12025	1555 (75)			
Roofing asphalt					
2-a (232°C)	34.1	12 (1)			
2-b (316°C)	12.9	10 (1)			
3-a (232°C)	34.2	12 (2)			
3-b (316°C)	128.3	10 (1)			
Paving asphalts (generated at 163°C- except as noted)					
4	16.34	24 (9)	49 (10)	16 (8)	29 (9)
5-a	16.37	22 (5)	21 (6)		22 (4)
5-b (221°C)	3.36	12 (10)	30 (12)	12 (5)	18 (13)
6	10.76	18 (2)	21 (3)		20 (2)
7	6.63	19 (3)	21 (3)		20 (2)
8	8.76	14 (2)	21 (2)		18 (2)
9	5.4	17 (3)	15 (3)		16 (2)

10	7.62	11 (2)	19 (3)		15 (2)
11	21.25	20 (4)	9 (3)		14 (3)
12	12.15	12 (2)	14 (2)		13 (2)
13	12.07	12 (6)	16 (5)	8 (5)	12 (3)
14	7.15	10 (2)	12 (2)		11 (1)
15	17.02	11 (3)	12 (3)		11 (2)
16-a (6 hr.)	7.18	13 (2)	10 (1)		11 (1)
16-b (2 hr.)		7 (2)	11 (2)		9 (2)
17-a (AC-10)	8.92	7 (1)			7 (1)
17-b (AC-20)	18.66	7 (1)	6 (1)		7 (1)
17-c (AC-30)	10.76	9 (1)	6 (2)		7 (1)
18	3.32	5 (2)	8 (2)		6 (2)

* Value is the sum of 18 PAH

Test substance : The following materials were used:

Two coal tar pitches representing ASTM Type I specification for roofing products

Two asphalts conforming to ASTM Type III roofing specifications. These represented different crude oil sources. They were identified as:
Asphalt No. 2 which was air-blown without the use of catalyst
Asphalt No. 3 which was air-blown using ferric chloride as catalyst

18 paving asphalts representing 14 different crude oil sources and various processing conditions.

Conclusion : The authors concluded that the asphalt fume condensates were weak to moderately mutagenic.
For the two roofing asphalts, mutagenic activity was unaffected by crude oil source, processing conditions or fume generation temperature.
For the paving asphalts derived from different crude oils, the mutagenicity indices differed over a five-fold range.

Reliability : (1) valid without restriction

(36)

Type : Modified Ames test
System of testing : S. typhimurium TA98
Metabolic activation : With
Year : 1990
GLP : No data
Test substance : Asphalts and their fumes

Result : Other bacterial mutagenicity studies (Ames test or modification of the Ames assay) have been conducted on asphalt fume condensates and all have shown similar supportive results and are not, therefore, described in detail here.
These studies have been reported by:

Reinke et al (2000)
De Meo et al (1998)
Kriech and Blackburn (1990)

A publication by Pasquini et al (1989) reports an Ames assay using S. typhimurium strains TA98 and TA100. The test was carried out on a DMSO extract of a whole asphalt and no mutagenic activity was found.

(15) (29) (41) (44)

Type	:	Mouse lymphoma assay
System of testing	:	L5178Y TK+/- mouse lymphoma cell line
Test concentration	:	0.061 to 1000 nl/ml
Metabolic activation	:	With and without
Year	:	1984
GLP	:	Yes
Test substance	:	acuum residue API sample 81-13 (See section 1.1.1.)

Method :

Assays were carried out with and without metabolic activation. The activation system used was an S9 fraction of Araclor-induced male mouse liver homogenate.

Prior to the assay, doses were selected by exposing the cultures of mouse lymphoma cells to a series of concentrations of the test material to determine its cytotoxicity.

Non-activation assay
Cultures of mouse lymphoma cells were exposed to the test material for 4 hours at concentrations that had been preselected on the basis of the results of the preliminary cytotoxicity study. The cells were then washed and placed in growth medium for two to three days to allow recovery, growth and expression of the induced TK-/- phenotype.
At the end of the expression period, 3×10^{6} cells for each selected dose were seeded onto soft agar plates with selection medium, and resistant (mutant) colonies were counted after 10 days incubation.
To determine the actual number of cells capable of forming colonies, a portion of the cell suspension was cloned in normal, nonselective, medium. The ratio of resistant colonies to total viable cell number is the mutant frequency.

Activation assay
The activation assay was run concurrently with the non-activation assay. The only difference was that the S9 fraction of mouse liver and the various cofactors was added during the 4 hour incubation period.

The solvent control was acetone.
The positive control substance for the non-activation assay was ethyl methane sulfonate and for the activation assay was dimethyl nitrosamine.

The criteria used in assessing the results of the assay were:

the minimum condition necessary to demonstrate mutagenesis for any given treatment is a mutant frequency that exceeds 150% of the concurrent background frequency by at least 10×10^{-6}.
The background frequency is defined as the average mutant frequency of the solvent and untreated controls.

The observation of a mutant frequency that meets the minimum criteria for a single treated culture within a range of assayed concentrations is not sufficient evidence.
The following test results must also be obtained:

A dose-related or toxicity-related increase must be observed. (Usually over three doses)

An increase in mutant frequency may be followed by only a small or

no further increase at higher concentrations or toxicities.

If an increase of about two times the minimum criterion or greater is observed for a single dose near the highest testable concentration, the test material shall be considered mutagenic.

Result : Two trials were carried out, and the results for each trial are summarized in the following two tables.

First trial

Test condition	**Rel. susp. growth (% of controls)**	**Total mutant colonies**	**Total viable**	**Mutant frequency (10 E^{-6} units)**
Non activation				
solvent	100	38	445	8.5
solvent	100	37	296	12.5
untreated	175.6	35	295	11.9
EMS 0.25 µl/ml	47.6	572	36	1588.9
test material				
62.5 nl/ml	158.9	23	146	15.8
125 nl/ml	131.8	34	171	19.9
250 nl/ml	176.3	13	192	6.8
500 nl/ml	152.9	39	247	15.8
1000 nl/ml	123.4	44	187	23.5
S9 activation				
solvent	100	97	307	31.6
solvent	100	122	319	38.2
untreated	88.1	60	213	28.2
DMN 0.3 µl/ml	57.1	138	53	260.4
test material				
62.5 nl/ml	66.1	107	252	42.5
125 nl/ml	100.4	119	206	57.8
250 nl/ml	97.3	105	152	69.1
500 nl/ml	84.3	164	166	98.8
1000 nl/ml	93.3	185	249	74.3

There was no evidence of mutagenic activity under non-activation conditions. However, with metabolic activation there was an indication of weak activity and a second trial using four doses in duplicate was carried out with activation only.
The results of the second trial confirmed those of the first and are summarized below.

Test condition	**Rel. susp. growth (% of controls)**	**Total mutant colonies**	**Total viable**	**Mutant frequency (10 E-6 units)**
solvent	100	89	236	37.7
solvent	100	86	267	32.2
untreated	114.3	68	262	26.6
DMN 0.3 µl/ml	50.2	175	52	336.5
DMN 0.3 µl/ml	26	181	50	362
test material				
700 nl/ml	47.6	214	214	100

700 nl/ml	48	183	215	85.1
800 nl/ml	40.1	162	208	77.9
800 nl/ml	73.1	167	200	83.5
900 nl/ml	58.4	175	149	117.4
900 nl/ml	70.1	166	194	85.6
1000 nl/ml	91.3	204	186	109.7
1000 nl/ml	106.3	147	178	82.6

The mutant frequencies were all elevated over the negative controls and all exceeded the criterion of 58.0 x 10^{-6} used to indicate mutagenic activity. The increases varied between 2.4 - 3.7-fold.

Reliability : (1) valid without restriction

(7)

Type : Mouse lymphoma assay
System of testing : L5178Y TK+/- mouse lymphoma cell line
Test concentration : 0.061 to 1000 nl/ml
Metabolic activation : With and without
Year : 1984
GLP : Yes
Test substance : Vacuum residue API sample 81-14 (See section 1.1.1.)

Result : Weakly mutagenic with metabolic activation.
Reliability : (1) valid without restriction

(8)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : 5 days
Doses : 0.3, 1.0 and 3.0 g/kg/day for five days
Year : 1984
GLP : Yes
Test substance : Vaccum residue API sample 81-13 (See section 1.1.1.)

Method : Test material was administered once daily by gavage as solutions in corn oil to groups of ten male and ten female rats at doses of 0.3, 1.0 and 3.0 g/kg/day for five days.
A negative control group of 10 rats of each sex received corn oil alone and the positive control group of ten rats of each sex received triethylenemelamine (TEM) as a single dose (1 mg/kg in 0.9% saline).
All animals were killed 6 hours after the last exposure to either test material, vehicle control or TEM.
Three hours prior to kill, all animals were given colchicine (4.0 mg/kg, intraperitoneally) to arrest cell division.
Bone marrow was aspirated from the bone and transferred to Hank's balanced salt solution. The marrow button was collected by centrifugation and resuspended in 0.075M KCl. Cells were fixed in methanol:acetic acid and slides were prepared and stained with Giemsa.
Slides were examined for chromosomal aberrations.
Routinely, 50 spreads were read for each animal. A mitotic index based on at least 500 cells was recorded. It was calculated by scoring the number of

cells in mitosis per 500 cells on each slide read.

Evaluation criteria and data interpretation
Gaps were not counted as significant aberrations.
Open breaks were considered as indicators of genetic damage, as were configurations resulting from the repair of breaks.
Number of aberrations per cell was considered as significant. Cells with more than one aberration were considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of the mutagenic potential.
In any event, the type of aberration, its frequency and its correlation to dose in a given time period were considered in evaluating a test article as being mutagenically positive or negative.

Statistical analysis employed a Student t-test.

Result : Many animals representing all treatment groups showed bilateral puffiness of upper eyelids. Necropsy resulted in a diagnosis of probably SDAV infection (sialodacryoadenitis), a common viral infection in rats.
The infection was not considered to have influenced the results of the assay.
The pooled results for males and females is shown in the following table.

	Neg control	Pos control	Test (mg/kg/day 0.3	1.0	3.0
No. animals	16	16	18	19	18
Total No. cells	755	455	843	818	857
No. structural aberrations	2	>956	3	4	3
No. numerical aberrations	8	16	4	8	8
% cells with 1 or more structural aberrations	0.3	47.5**	0.4	0.5	0.4
% cells with 2 or more structural aberrations	0	29.9**	0	0	0
%Mitotic Index	3.5	0.6	3.5	3.7	3.6

The authors concluded that the test material was negative in inducing chromosomal aberrations in rat bone marrow cells in this assay.

Reliability : (1) valid without restriction

(7)

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : Once each day for 5 days
Doses : 0.4, 1.3 and 4 g/kg/day
Result : Negative
Year : 1984
GLP : Yes
Test substance : Vacuum residue API sample 81-14 (See section 1.1.1.)

Method : Test material was administered once daily by gavage as solutions in corn oil to groups of ten male and ten female adult Sprague Dawley rats at

doses of 0.4, 1.3 and 4.0 g/kg/day for five days.
A negative control group of 10 rats of each sex received corn oil alone and two positive control groups of ten rats of each sex received triethylenemelamine (TEM) as a single dose of either 0.75 or 1.0 mg/kg in 0.9% saline) .
All animals were killed 6 hours after the last exposure to either test material, vehicle control or TEM. Three hours prior to kill, all animals were given colchicine (4.0 mg/kg, intraperitoneally) to arrest cell division.
Bone marrow was aspirated from the bone and transferred to Hank's balanced salt solution. The marrow button was collected by centrifugation and resuspended in 0.075M KCl. Cells were fixed in methanol:acetic acid and slides were prepared and stained with Giemsa. Slides were examined for chromosomal aberrations. Routinely, 50 spreads were read for each animal. A mitotic index based on at least 500 cells was recorded. It was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.

Evaluation criteria and data interpretation
Gaps were not counted as significant aberrations.
Open breaks were considered as indicators of genetic damage, as were configurations resulting from the repair of breaks. Number of aberrations per cell was considered as significant. Cells with more than one aberration were considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number were also considered in the evaluation of the mutagenic potential. In any event, the type of aberration, its frequency and its correlation to dose in a given time period were considered in evaluating a test article as being mutagenically positive or negative.

Statistical analysis employed a Student t-test.

Result : No clinical signs of toxicity were reported following exposure to the test material.
The pooled results for males and females are shown in the following table.

	Controls			**Test (mg/kg/day)**		
	-ve	**+ve 0.75 (mg/kg)**	**+ve 1.0**	**0.4 (mg/kg)**	**1.3**	**4.0**
No. animals	16	7	17	18	17	15
Total No. cells	759	182	350	792	820	750
No. structural aberrations	4	>359**	>687**	3	3	5
No. numerical aberrations	13	6	8	20	12	14
% cells with 1 or more structural aberrations	0.4	36.3**	32.9**	0.4	0.4	0.5
% cells with 2 or more structural aberrations	0.1	28.0**	26.6**	0	0	0.1
%Mitotic Index	5.9	0.6	0.3	6.3	6.0	6.4

The authors concluded that the test material was negative in inducing chromosomal aberrations in rat bone marrow cells in this assay.

Reliability : (1) valid without restriction

(8)

Type	:	DNA Adduct formation
Species	:	Rat
Sex	:	Male
Strain	:	CD
Route of admin.	:	Intratracheal instillation
Doses	:	2250, 500 & 1000 mg/kg
Year	:	1998
GLP	:	No data
Test substance	:	Condensed asphalt fumes

Method : Three male CD rats (4-6 weeks old) were instilled (tracheal) with solvent (negative control), benzo(a)pyrene (positive control) or test material at three dose levels three times every 8 hours for a total of 3 doses.
Six hours after the third dose, animals were anesthetized and lung tissues were harvested and were cut into small pieces for the isolation of DNA.
Blood was also collected and after treatment with EDTA, the white blood cells were separated by density centrifugation.
DNA was isolated from rat lung cells using a standard procedure using a phenol/ethnol extraction and purification with RNAse digestion.
The procedures for postlabeling DNA have been described elsewhere.
^{32}P-labelled adducts were separated by 2-dimensional chromatography and were visualized by autoradiography.
The separated adducts were relatively quantified and adduct levels were calculated.
A 2-tailed Student's t-test was used to analyze the difference in the DNA adduct levels between the control and treated groups.

Remark : This study was carried out with a view to identifying a suitable biomarker for exposure to asphalt fumes.

Result : The number of adducts identified following the various treatments are shown in the following table.

Dose	**Adduct spot**	**Total adducts/10^8 nucleotides (mean±SD)**	
		Type I asphalt	**Type III asphalt**
Control(DMSO 3 ml/kg)		4.9 ± 4.0	5.8 ± 2.7
250 mg/kg	1	25.8 ± 16.3	24.3 ± 4.5*
500 mg/kg	1	49.0 ±4.9*	33.7 ± 9.2*
1000 mg/kg	1	71.0 ± 3.5*	67.8 ± 6.7*
B(a)P 10 mg/kg	1	46.2 ± 1.9*	44.1 ± 5.6*

* P<0.01

Although clear adduct formation was detected in WBC of rats exposed to B(a)P, no adducts were found in the WBC of rats treated with fume condensate.
In conclusion, DNA adducts did occur in lung cells of rats that had been instilled tracheally with fume condensates of either Type I or Type III roofing asphalt. In contrast, no adducts were found in the WBC of the same animals.

Test substance : Type I and Type III roofing asphalts were used in the study. The fume condensates were prepared by heating small pieces of the asphalts to 316 ± 10 °C in round bottomed flasks. The fumes that were generated were collected in glass impingers in cryotraps and organic solvents (50:50 mixture of cyclohexane/acetone). Collected materials from all impingers were combined and separated into water and organic phases. Water and solvents were removed and the condensates from both phases were combined.

(43)

5.7 CARCINOGENICITY

Species : Mouse
Route of admin. : Skin and inhalation
Test substance : Asphalts, various

Result : Many carcinogenicity studies have been reported for various types of asphalt.
The studies have included:

- skin painting studies of whole asphalts and of extracts or solutions of whole asphalts
- Skin painting studies of condensed asphalt fumes
- Inhalation studies of asphalt fumes

The studies are presented in a summarized form in the attached table. The attachment also includes the references to the studies. These data have also been summarized previously by CONCAWE (CONCAWE 1992).

In general, whole asphalts have been shown to be non-carcinogenic when applied undiluted to the skin (but heated to assist application). When applied as solutions in organic solvents the asphalts have been shown to be weakly carcinogenic.

Inhalation of bitumen fumes has not demonstrated a carcinogenic effect.

Condensed fumes have been shown to cause skin tumors in mice. However, the use of organic solvent for skin application and higher than normal temperatures to generate the fumes casts some doubt on the validity or relevance of the results.

Attached document : Carcinogenicity studies.doc

(27)

(1) American Petroleum Institute. (1987).
Comprehensive Analytical Analysis of API Generic Refinery Streams. API, Washington, DC.

(2) API (1982)
Acute toxicity studies
Vacuum residuum Sample 81-13
American Petroleum Institute Med. Res. Publ. 30-31987

(3) API (1983)
28-day dermal toxicity study in the rabbit. Vacuum residuum API sample 81-13
American Petoleum Institute Med. Res. Publ. 30-32852

(4) API (1983)
28-day dermal toxicity study in the rabbit. Vacuum residuum API sample 81-14
American Petoleum Institute Med. Res. Publ. 30-32853

(5) API (1984)
Dermal sensitization study in Guinea pigs, Closed patch technique. Vacuum residuum API sample 81-13
American Petroleum Institute Med. Res. Publ. 31-31415

(6) API (1984)
Dermal sensitization study in Guinea pigs, Closed patch technique. Vacuum residuum API sample 81-14
American Petroleum Institute Med. Res. Publ. 31-31416

(7) API (1984)
Mutagenicity evaluation studies in the rat bone marrow cytogenetic assay and in the mouse lymphoma forward mutation assay
Vacuum residuum API Sample 81-13
American Petroleum Institute Res. Rep. 31-30614

(8) API (1984)
Mutagenicity evaluation studies in the rat bone marrow cytogenetic assay and in the mouse lymphoma forward mutation assay
Vacuum residuum API Sample 81-14
American Petroleum Institute Res. Rep. 31-30615

(9) API (2003)
Test plan and robust summary submission for HPV category: Lubricating Oil Basestocks
URL: http//www.epa.gov/opptintr/chemrtk/viewsrch.htm

(10) Asphalt Institute (2003)
Evaluation of hot mix asphalt for leachability
Asphalt Institute, Lexington, Kentucky
Asphalt Institiute website url:
http://www.asphaltinstitute.org

(11) Asphalt Institute (2003)
Oregon and Washington Fish Hatcheries Lined with Asphalt. Asphalt Institute, Lexington, Kentucky.
Asphalt Institute web site url
http://www.asphaltinstitute.org

(12) Asphalt Institute. (2003)
Leachability of Asphalt and Concrete Pavements.
Asphalt Institute, Lexington, Kentucky.
Asphalt Institute web site url
http://www.asphaltinstitute.org

(13) ASTM (2000)
Standard test method for softening point of bitumen (Ring and Ball Apparatus)
ASTM, Conshohocken, PA

(14) Atkinson, R. (1990).
Gas-phase tropospheric chemistry of organic compounds: A review. Atmos. Environ. 24A:1-41.

(15) Blackburn, G. R. and Kreich, A. J. (1990)
Status report on industry-sponsored toxicology and chemical testing of asphalts and asphalt fume condensates.
Heritage Research Group. Indianapolis, Indiana

(16) BP International Limited. (1990).
The Acute Toxicity to Rainbow Trout (Salmo gairdneri). Project No. 301/65; Report No. AT301/044.
Study performed by Safepharm Laboratories Ltd, Derby, U.K.

(17) BP International Limited. 1990.
Assessment of the Algistatic Effect on Scenedesmus subspicatus. Project No. 301/72.
Study performed by Safepharm Laboratories Ltd, Derby, U.K.

(18) BP Oil Europe. (1994).
PSG 1857: Algal inhibition test. Project Number 599/42, conducted by Safepharm Laboratories Limited, Derby, U.K.

(19) BP Oil Europe. (1994).
The acute toxicity of PSG 1857 to Daphnia magna.
Project Number 599/43,
conducted by Safepharm Laboratories Limited, Derby, U.K.

(20) BP Oil Europe. (1994).
The acute toxicity of PSG 1857 to rainbow trout (Oncorhynchus mykiss). Project Number 599/44,
conducted by Safepharm Laboratories Limited, Derby, U.K.

(21) BP Oil Europe. (1995).
Assessment of the Effect of PSG 1860 on the Reproduction of Daphnia magna. Project Number 692/12,
conducted by Safepharm Laboratories Limited, Derby, U.K.

(22) BP Oil Europe. (1995).
Daphnia magna Reproduction Test. SPL Project No. 692/038. Study performed by Safepharm Laboratories Ltd, Derby, U.K.

(23) Brandt, H.C.A, and P.C. De Groot (2001)
Aqueous leaching of polycyclic aromatic hydrocarbons from bitumen and asphalt.
Wat. Res. 35(17):4200-4207.

(24) Buckler, D. R. and Granato, G. E. (1999).
Assessing Biological Effects from Highway-Runoff Constituents.
Report No. 99-240.
United States Geological Survey, Northborough, Massachusetts.

(25) CONCAWE (1997)
Lubricating oil basestocks
Product dossier No. 97/108

(26) CONCAWE (2001)
Environmental classification of petroleum substances - Summary data and rationale
Report No. 01/54

(27) CONCAWE. (1992).
Bitumens and Bitumen Derivatives.
Product Dossier No. 92/104,
CONCAWE, Brussels.

(28) Cundell, A.M. and R.W. Traxler. (1973).
Microbial degradation of petroleum at low temperature.
Mar. Poll. Bull. 4(8):125-127.

(29) De Meo, M., Genevois, C., Brandt, H., Laget, M., Bartsch, H. and Castegnaro, M. (1996)
In vitro studies of the genotoxic effects of bitumen and coal-tar fume condensates: comparison of data obtained by mutagenicity testing and DNA adduct analysis by 32P-postlabelling
Chem. Biol. Interact. Vol 101, No. 2, pp 73-88

(30) EPA. (2000).
Estimation Programs Interface for Windows (EPIWIN).
United States Environmental Protection Agency, Washington, DC.

(31) Fraunhofer (2000)
Acute inhalation toxicity study of 100 mg/m bitumen fumes in Wistar (WU) rats
Fraunhofer ITA Study No. 02G00012

(32) Fraunhofer (2001)
13 Week inhalation toxicity study of bitumen fumes in Wistar (WU) rats
Fraunhofer ITA study No. 02G01005

(33) Harris, J.C. (1982).
Rate of Hydrolysis. In: Handbook of Chemical Property Estimation Methods.
W.J. Lyman, W.F. Reehl and D.H. Rosenblatt, eds. McGraw-Hill Book Company, New York, NY.

(34) IARC (1985)
IARC monographs on the evaluation of carcinogenic risks to humans. Volume 35., Polynuclear aromatic compounds, Part 4:
bitumens, coal-tars and derived products, shale-oils and soots.
International Agency for Research on Cancer

(35) IARC (1987)
Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1-42 Supplement No. 7
International Agency for Research on Cancer

(36) Machado, M. L., Beatty, P. W., Fetzer, J. C., Glickman, A. H. and McGinnis, E. L. (1993)
Evaluation of the relationship between PAH content and mutagenic activity of fumes from roofing and paving asphalts and coal tar pitch
Fund. Appl. Toxicol. Vol 21, pp 492-499

(37) Monarca, S., Pasquini, R., Scassellati Sforzolini, G., Savino, A., Bauleo, F. A. and Angeli, G. (1987)
Environmental monitoring of mutagenic/carcinogenic hazards during road paving operations with bitumens
Int. Arch. Occup. Environ. Health Vol 59, pp 393-402

(38) NIOSH (National Institute for Occupational Safety and Health). (2000).
Hazard Review: Health Effects of Occupational Exposure to Asphalt.
U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health , Cincinnati, Ohio.

(39) NIOSH (National Institute for Occupational Safety and Health). (2001).
Asphalt Fume Exposures During the Manufacture of Asphalt Roofing Products: Current Practices for Reducing Exposures. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health , Cincinnati, Ohio.

(40) OECD (Organisation for Economic Co-operation and Development. (1993).
OECD Guidelines for Testing of Chemicals. OECD, Paris, France.

(41) Pasquini, R., Taningher, M., Moarca, S., Pala, M. and Angeli, G. (1989)
Chemical composition and genotoxic activity of petroleum derivatives collected in two working environments.
J. Tox and Env. health Vol 27, pp 225-238

(42) Phillips, U.A., and R.W. Traxler. (1963).
Microbial Degradation of Asphalt.
Appl. Microbio. 11:235-238.

(43) Qian, H. -W., Ong, T., Nath, G. and Whong, W. -Z (1998)
Induction of DNA adducts in vivo in rat lung cells by fume condensates of roofing asphalt.
Teratogenesis, Carcinogenesis and Mutagenesis, Vol 18, pp 131-140

(44) Reinke, G., Swanson, M., Paustenbach, D and Beach, J. (2000)
Chemical and mutagenic properties of asphalt fume condensates generated under laboratory and field conditions.
Mutat. Res. Vol 469, No. 1, pp 41-50

(45) Robinson, M., Bull, R. J., Munch,J. and Meier, J (1984)
Comparative carcinogenic and mutagenic activity of coal tar and petroleum asphalt paints used in potable water supply systems
J. App. Toxicol. Vol 4, No. 1, pp 49-56

(46) Shell Research Ltd. (1988).
Oils: Acute toxicity of four oils to Daphnia magna and Gammarus pulex.
SBGR.88.075.

(47) USEPA. (1985).
Oil and Hazardous Materials/Technical Assistance Data System (OHM/TADS).

(48) ZoBell, C.E. and M.A. Molecke (1978)
Survey of Microbial Degradation of Asphalts with Notes on Relationship to Nuclear Waste Management.
Report No. SAND78-1371,
Scripps Institution of Oceanography, La Jolla, California. 31 pp.

DERMAL CARCINOGENICITY STUDIES

MATERIAL TESTED	TREATMENT	DURATION	RESULTS	REFERENCE
Penetration asphalts				
Steam refined (1 sample)	Undiluted (heated)	21 months	5/63 mice with skin tumors 21/63 mice survived study	Simmers (1965)
Road bitumen (4 samples)	Diluted with acetone (concentration unspecified) Application twice/week	2 years	0/100, 2/50, 1/50 & 0/50 mice with skin tumors	Hueper & Payne (1960)
Penetration bitumens (4 samples)	40% in benzene Application once/week	19 months	9/52, 4/47, 2/50 &2/50 mice with skin tumors	Kireeva (1968)
Penetration bitumen (8 samples)	10% in benzene Application twice/week	>81 weeks	Highest incidence 7% Lowest incidence 0% Overall incidence 2.7%	Walcave et al (1971)
Penetration bitumen (1 sample)	30% in mineral oil Application twice/week	24 months	0/50 mice	McGowan et al (1992)
Hard Asphalts				
Bitumen paint (1 sample)	60% bitumen in mineral spirit Application once/week	30 weeks	1/40 mice with skin tumor	Robinson et al (1984)
Oxidized bitumens				
Air blown bitumen (1 Sample)	Undiluted (heated) Application 1 to 3 times/week	21 months	1/50 mice with skin tumor 10 mice survived	
Air blown bitumen (1 Sample)	90% in toluene Application three times/week	2 Years	9/20 mice with skin tumors	Simmers (1965)
Roofing bitumen (1 Sample)	Diluted in acetone, concentration unspecified Application twice/week	2 Years	1/50 mice with skin tumors	Hueper & Payne (1960)
Roofing bitumen (1 sample)	50% in toluene Application twice/week	80 weeks	0/50 mice with skin tumors	Emmet et al (1981)
Roofing bitumen (1 sample)	50% in acetone/cyclohexane Application twice/week	2 Years	3/30 mice with skin tumors	Sivak et al (1989)
Mixed penetration & Oxidized bitumens				
Mixture of 6 air-blown and steam-refined bitumens	Diluted with benzene, concentration unspecified Application twice/week	Time unspecified, but > 54 weeks	17/68 mice with skin tumors	Simmers et al (1959)
Thermally cracked bitumens				
Oxidized residue bitumen (2 samples)	40% in benzene Application once weekly	19 months	9/49 & 4/42 with skin tumors	Kireeva (1968)
Vacuum residuum				
2 samples API 81-13 & 81-14	Diluted in toluene 50µl twice/week	130 weeks	5/50 & 2/50 mice with skin tumors. Mean latenc 113 & 120 weeks	API (1989)

INHALATION CARCINOGENICITY STUDIES

Oxidized bitumen (1 sample)	Fumes generated at 250-275°F Exposure 5 hr/day, 4 days/week 65 Bethesda strrn rats 13 Guinea pigs used	2 Years	No lung tumors, but extensive fibrosing pneumonitis was observed in rats	Hueper & Payne (1960)
Mixture of 6 penetration grades and oxidized bitumens	20 C57 mice exposed 30 mins/day, five days/week Aerosol generated at 250°F	17 months	1 animal with lung adenoma	Simmers (1964)
Mixture of 6 penetration grades and oxidized bitumens	30 C57 mice exposed 6-7½hrs/dayfive days/week Smoke generated at 250°F	21 months	Bronchitis, loss of bronchial coilia, epithelial atrophy, necrosis, pneumonitis No lung tumors observed	Simmers (1964)

SKIN APPLICATION OF CONDENSED FUMES

Type I & Type III asphalt	Fumes generated at 450 & 601°F Application twice/week as 50% solution in cyclohexane/acetone. Some animals also exposed to UV light CD 1 and C3H mice used	Up to 72 weeks	C3H more sensitive than CD-1. Greater tumor response from fume generated at the higher temperature.	Niemeier et al (1988)
Type III asphalt	Fumes generated same method as by Niemeier but at 601°F only C3H and Sencar mice used Sample applied twice weekly	104 weeks	C3H mouse 20/30 mice with tumors Sencar : 14/30 mice with tumors	Sivak et al (1989, 1997)

REFERENCES

American Petroleum Institute (1989)
Chronic dermal carcinogenesis assay in C3H/HeJ male mice using vacuum residuum samples, API 81-13 and API 81-14
Research report No. 36-32643

Emmet, E. A. et al. (1981)
A carcinogenic bioassay of certain roofing materials
Am. J. Ind. Med. 2, 59-64

Hueper, W. C. and Payne, W. W. (1960)
Carcinogenic studies on petroleum asphalt, cooling oil and coal tar.
Arch. Path., 70, (3), 372-384

Kireeva, I. S. (1968)
Carcinogenic properties of coal tar pitch and petroleum asphalts used as binders for coal briquettes
Hyg. Sanit., 33, 35-40

McGowan, C. et al. (1992)
Lack of carcinogenic and mutagenic activity with asphalt products
Abstract 1485.
The Toxicologist, 12, (1), 379

Niemeier et al (1988)
A comparison of the skin carcinogenicity of condensed roofing asphalt and coal tar pitch fumes.
In: Cooke, M and Dennis, A.J. eds., Polynuclear Aromatic Hydrocarbons; A decade of progress.
10th International symposium on Polynuclear Aromatic Hydrocarbons.
Batelle press pp 609-647

Robinson, M. et al. (1984)
Comparative carcinogenic and mutagenic activity of coal tar and petroleum asphalt paints used in potable water supply systems
J. Appl. Toxicol., 4, (1), 49-56

Simmers , M. H. et al. (1959)
Carcinogenic effects of petroleum asphalt
Proc. Soc. Exp. Biol. Med, 101, 266-268

Simmers, M. H. et al (1964)
Petroleum asphalt inhalation by mice
Arch. Env. Health. 9, 727-734

Simmers, M. H. et al. (1965)
Cancers from air-refined and steam-refined asphalt
Ind. Med. Surg, 34, 255-261

Sivak et al. (1989)
Assessment of the cocarcinogenic/promoting activity of asphalt fumes
NIOSH No. 200-83-2612
NTIS Publication PB-91-110-213

Sivak et al. (1997)
Skin carcinogenicity of condensed asphalt roofing fumes and their fractions following dermal applications to mice
Cancer letters, 74, 614-615

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Skin tumorigenesis in mice by petroleum asphalts and coal tar pitches of known polynuclear aromatic hydrocarbon content.
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DERMAL CARCINOGENICITY STUDIES

Material tested	Treatment	Duration	Results	Reference
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